SPECTROSCOPIC METHODS FOR BODY FLUID AGE DETERMINATION

This application is a national stage application under 35 U.S.C. § 371 of PCT Application No. PCT/US16/44837, filed Jul. 29, 2016, which claims the priority benefit of U.S. Provisional Patent Application Ser. No. 62/199,063, filed Jul. 30, 2015, the disclosure of which is hereby incorporated by reference in its entirety.

This invention was made with government support under Award No. 2011-DN-BX-K551 and 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.). The government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to a method of determining the age of body fluid.

BACKGROUND OF THE INVENTION

Effective forensic crime scene processing relies on a rapid, informative, and high throughput investigation starting from evidence discovery and collection at the scene all the way through to analysis in the laboratory (Lee et al., "Crime Scene Reconstruction," *Henry Lee's Crime Scene Handbook*. Elsevier Academic Press: San Diego, Calif. pp. 271-98 (2001)). Especially in cases of violent crimes, such as assaults, murders, or homicides, blood can provide investigators with very critical information (Castro et al., "Review: Biological Evidence Collection and Forensic Blood Identification," (2013); James et al., "Principles of Bloodstain Pattern Analysis: Theory and Practice," CRC Press (2005); Bevel et al., "Bloodstain Pattern Analysis with an Introduction to Crime Scene Reconstruction," CRC Press (2008)). Knowing the age, or time since deposition (TSD), of a bloodstain can assist investigations in at least two ways: it can establish when a crime occurred, and it can discriminate bloodstains that relate to the crime from those that are extraneous. Primarily for these reasons, it has been a major goal in the field to establish a technique for determining the TSD of bloodstains accurately (Bremmer et al., "Forensic Quest for Age Determination of Bloodstains," *Forensic Sci. Int* 216(1-3):1-11 (2012)).

Various presumptive (Spalding, R. P., "Identification and Characterization of Blood and Bloodstains," In: James et al., Eds., *Forensic Science: An Introduction to Scientific and Investigative Techniques*, CRC Press:Boca Raton, pp. 181-201 (2009); Kobilinsky, L., "Forensic Chemistry Handbook," John Wiley & Sons (2012); Vandenberg et al., "The Use of Polilight in the Detection of Seminal Fluid, Saliva, and Bloodstains and Comparison with Conventional Chemical-Based Screening Tests," *J. Forensic Sci.* 51(2):361-370 (2006); Webb et al., "A Comparison of the Presumptive Luminol Test for Blood with Four Non-Chemiluminescent Forensic Techniques," *Luminescence* 21:214-20 (2006); Lin et al., "Forensic Applications of Infrared Imaging for the Detection and Recording of Latent Evidence," *J. Forensic Sci.* 52(5):1148-50 (2007); Johnston et al., "Comparison of Presumptive Blood Test Kits Including Hexagon OBTI," *J. Forensic Sci.* 53(3):687-9 (2008); Brooke et al., "Multimode Imaging in the Thermal Infrared for Chemical Contrast Enhancement. Part 3: Visualizing Blood on Fabrics," *Anal. Chem.* 82(20):8427-31 (2010)) and confirmatory (Dixon et al., "A Scanning Electron Microscope Study of Dried Blood," *J. Forensic Sci.* 21(4):797-803 (1976); Sottolano et al., "An Improved Technique for the Preparation of Teichman and Takayama Crystals from Blood," *The Microscope* 28(2):41-6 (1980); Kotowski et al., "The Use of Microspectrophotometry to Characterize Microscopic Amounts of Blood," *J. Forensic Sci.* 31(3):1079-85 (1986); Kashyap, V. K., "A Simple Immunosorbent Assay for Detection of Human Blood," *J. Immunoassay* 10(4):315-24 (1989); U.S. Pat. No. 7,270,983 to Ballantyne et al.; Bauer, M., "RNA in Forensic Science," *Forensic Sci. Int. Genet.* 1:69-74 (2007); Bauer et al., "Identification of Menstrual Blood by Real Time RT-PCR: Technical Improvements and the Practical Value of Negative Test Results," *Forensic Sci. Int* 174:55-9 (2008); Haas et al., "mRNA Profiling for Body Fluid Identification by Reverse Transcription Endpoint PCR and Real-time PCR," *Forensic Sci. Int. Genet.* 3(2):80-8 (2009)) tests can be used to check for the presence of blood, but they each have considerable disadvantages. In a 2009 review article of forensic body fluid testing, Virkler et al. stated that, although highly desired, no single in-field method exists that is non-destructive, applicable to multiple body fluids, and provides a confirmatory result (Virkler et al., "Analysis of Body Fluids for Forensic Purposes: From Laboratory Testing to Non-Destructive Rapid Confirmatory Identification at a Crime Scene," *Forensic Sci. Int* 188(1-3):1-17 (2009)). While it is preferable to conclusively identify a stain as blood before DNA testing, this is almost never done in practice due to the lack of time and money (Kobilinsky, L., "Forensic Chemistry Handbook," John Wiley & Sons (2012)). Some of the problems associated with current identification methods have been addressed by applying Raman spectroscopy with advanced statistical methods. The use of this approach allowed to differentiate between six body fluids (Sikirzhytski et al., "Multidimensional Raman Spectroscopic Signatures as a Tool for Forensic Identification of Body Fluid Traces: A Review," *Appl. Spectrosc.* 65(11):1223-32 (2011)) and to create multidimensional spectroscopic signatures for blood (Virkler et al., "Raman Spectroscopic Signature of Blood and its Potential Application to Forensic Body Fluid Identification," *Anal. Bioanal. Chem.* 396(1):525-34 (2010); McLaughlin et al., "A Modified Raman Multidimensional Spectroscopic Signature of Blood to Account for the Effect of Laser Power," *Forensic Sci. Int* 240:88-94 (2014)), saliva (Virkler et al., "Forensic Body Fluid Identification: The Raman Spectroscopic Signature of Saliva," *Analyst* 135(3):512-7 (2010)), semen (Virkler et al., "Raman Spectroscopic Signature of Semen and its Potential Application to Forensic Body Fluid Identification," *Forensic Sci. Int* 193(1-3):56-62 (2009)), sweat (Sikirzhytski et al., "Multidimensional Raman Spectroscopic Signature of Sweat and its Potential Application to Forensic Body Fluid Identification," *Anal. Chim. Acta.* 718 (0):78-83 (2012)), and vaginal fluid (Sikirzhytskaya et al., "Raman Spectroscopic Signature of Vaginal Fluid and its Potential Application in Forensic Body Fluid Identification," *Forensic Sci. Int* 216(1-3):44-8 (2012)), which took into account dry samples' heterogeneity and donor variations. Differentiation between human and animal blood (Virkler et al., "Blood Species Identification for Forensic Purposes Using Raman Spectroscopy Combined with Advanced Statistical Analysis," *Anal. Chem.* 81(18):7773-7 (2009); McLaughlin et al., "Discrimination of Human and Animal Blood Traces via Raman Spectroscopy," *Forensic Sci Int* 238(0):91-5 (2014); McLaughlin et al., "Raman Spectroscopy of Blood for Species Identification," *Anal. Chem.* 86(23):11628-33 (2014)) as well as peripheral and menstrual blood (Sikirzhytskaya et al., "Raman Spectroscopy Coupled with Advanced Statistics for Differentiating Menstrual and